PHYSIOLOGY.—*Studies on the oxygen consumption of* Australorbis glabratus *eggs.*

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The possibility has been pointed out that studies on the physiology of the intermediate hosts of schistosomiasis may yield clues to the development of chemical control measures, (von Brand, Nolan, and Mann, 1948). This reasoning led to extensive studies on snails by several investigators (Mehlman and von Brand, 1951; von Brand and Mehlman, 1953; von Brand, McMahon, and Nolan, 1955; Newton and von Brand, 1955; von Brand, 1955). They investigated the anaerobic and postanaerobic metabolism, the relation between the pre- and postanaerobic oxygen consumption and the oxygen tension, the effect of temperature and tissue hydration on anaerobic survival, and physiological differences between geographical strains. Such studies led to investigations of metabolic pathways potentially vulnerable to chemical attack. Weinbach (1952, 1953) studied the intermediate metabolism of *Australorbis glabratus*, and subsequently the mechanism by which pentachlorophenol kills snails was elucidated (Weinbach, 1954, 1956, Weinbach and Nolan, 1956).

In marked contrast to these studies on adult snails is the complete lack of knowledge concerning the metabolism of snail eggs and the mode of action of chemicals on them. Studies on eggs of other animals suggested that certain compounds such as di and trihalophenols block cleavage of fertilized *Arbacia* eggs (Clowes et al., 1950). It seems then possible that chemical control of the developing eggs of disease-transmitting snails may help in the control of certain parasitic diseases. To lay a foundation towards such an approach, a study of the respiration of *Australorbis glabratus* eggs was undertaken and is reported below.

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MATERIAL AND METHODS

The eggs were obtained from *Australorbis glabratus*, laboratory reared from Venezuelan stock. Twenty-five to thirty adult specimens were kept at room temperature (approx. 25°C) in jars containing 3 to 4 liter dechlorinated tapwater which was renewed once a week. The snails were fed abundantly with lettuce leaves and calcium carbonate was added to the water occasionally. Eight such jars were lined with glass slides on which the snails deposited the egg capsules. Egg clutches were collected daily at 9 a.m. thus permitting a rigorous control of their age. They were carefully removed from the slides with the help of a razor blade and placed in open dishes containing 200 to 300 ml water. Most of the eggs maintained in this way appeared to develop normally and only 5 to 10 percent died before hatching time. Dead eggs could, after some experience, be recognized even macroscopically, at least in the later stages of development. They usually became moldy and heavily contaminated with bacteria and were of course discarded. A further check, especially important with the young stages (zero and one day), was provided by examining the egg capsules under an entomological microscope.

The rate of oxygen consumption of the eggs was studied by means of Warburg manometers equipped with flasks of approximately 6 ml capacity containing 1 ml of dechlorinated tap water as respiratory medium. The carbon dioxide was absorbed in the customary manner by means of 10 percent KOH. An equilibration period of 30 minutes was allowed, and readings were taken at 30 minute intervals for 2 to 3 hours. The manometers were shaken with an amplitude of 4 cm 100 times per minute. The temperature in all experiments was 28°C. The data were calculated per egg. In order to minimize errors, the eggs contained in each egg capsule used in a given experiment were counted both before being introduced into the Warburg flask and at the end of the experiment.

Because the respiratory rate was higher in older eggs than in young ones, the number of clutches, that is of eggs, had to be varied. In most experiments with 0 to 2 day-old eggs, 30 egg capsules were used per flask, while 10 to 15 clutches containing older embryos sufficed to give adequate manometer changes. When it was desired to follow a certain group of clutches during its entire development, the 30 egg clutches used per flask during the first days were divided into smaller lots during the later days, and all these lots were tested on the same days separately, when possible. The egg clutches were introduced into the flasks individually by means of a very fine spatula and at the end of the experiment, they were washed out by means of a little water. Although these manipulations were done with great care, an occasional injury to an egg clutch could not always be avoided. Any damaged clutch was rejected.

RESULTS AND DISCUSSION

Data on the embryology of various species of fresh water snails are available in the literature (*Lymnaea* spp., Lankester, 1874, Crabb, 1927; *Stagnicola*, Lowrance, 1934; various Planorbidae, Holmes, 1900, Roney 1943, Baker, 1945), but no information concerning *Australorbis glabratus* specifically has come to our attention. We therefore record here briefly some pertinent observations.

The number of eggs per egg capsule is quite variable. It varied in the 855 clutches used in the present study from 5 to 58. But it should be pointed out that clutches containing only 2 to 3 eggs are frequently deposited by very young snails, while old specimens occasionally lay capsules containing over 60 eggs. The individual eggs are enclosed by an inner capsule. In it, the ovum proper usually occupies an eccentric position, although it can also be located centrally. Inner capsules containing two ovas or none, occur occasionally. The inner capsule, are arranged in a matrix in irregular, or sometimes parallel rows (Fig. 1 (1, 2)) and are bounded by a thin membrane.

Embryos allowed to develop at room temperature of about 25°C started hatching on the 6th to 8th day after oviposition. Increasing the temperature slightly increased the number of young snails hatching on the 6th day. Decreasing the temperature by 3 to 5°C retarded the development; the first hatchings occurred as late as the 9th or 10th day. There was some variation in the hatching time even among snails of a single egg clutch; the first ones could, for example, hatch on the 6th day with the remainder following during the next 2 to 3 days.

A detailed study of the embryology of *Australorbis* was not done. However, we did make sufficient observations to indicate that this snail follows the usual planorbid pattern. Representative stages are shown in Fig. 1 (3 to 9). They show the main stages used during our determinations of the oxygen consumption on the following days: 0, 1, 2, 4, 5, and 6, and, since taken at the same magnification, can serve as indication of the increase in living substance. The stage reached on day 3, although tested for oxygen consumption, was not photographed; it corresponded approximately to pre-veliger stage. Of some interest in connection with our respiratory studies is the motility of the embryos. First rotation of the developing ova occurred on day 2. On day 4 the embryos were already very active, moving actively around within the inner capsule; at this stage the heart beat was clearly visible. During the last day of development some variation in motility was noticed. Some of the now fully developed young snails were very active and were escaping the egg clutch. Others were more quiescent, and these required one or two days more to leave the clutch.

Two independent sets of experiments were conducted in which the rate of oxygen con-

FIG. 1.—Representative stages in the development of the eggs of *Australorbis glabratus*: (*1*) Complete egg clutch, 0 day, showing the arrangement of the inner capsules within the clutch, 10 ×. (*2*) Complete egg clutch, 6th day, showing the growth of the embryo, 10 ×. (*3*) Single egg, 0 day, cleavage stage, 90 ×. (*4*) Single egg, 1st day, probably trochophore stage, 90 ×. (*5*) Single egg, 2 days, post-trochophore stage, 90 ×. (*6*) Single egg, 3 days, veliger stage, 90 ×. (*7*) Single egg, 5 days, well developed embryo within the shell, 90 ×. (*8*) Freshly hatched snail in water, 90 ×. (*9*) Freshly hatched snail in air, retracted into the shell. 90 ×.

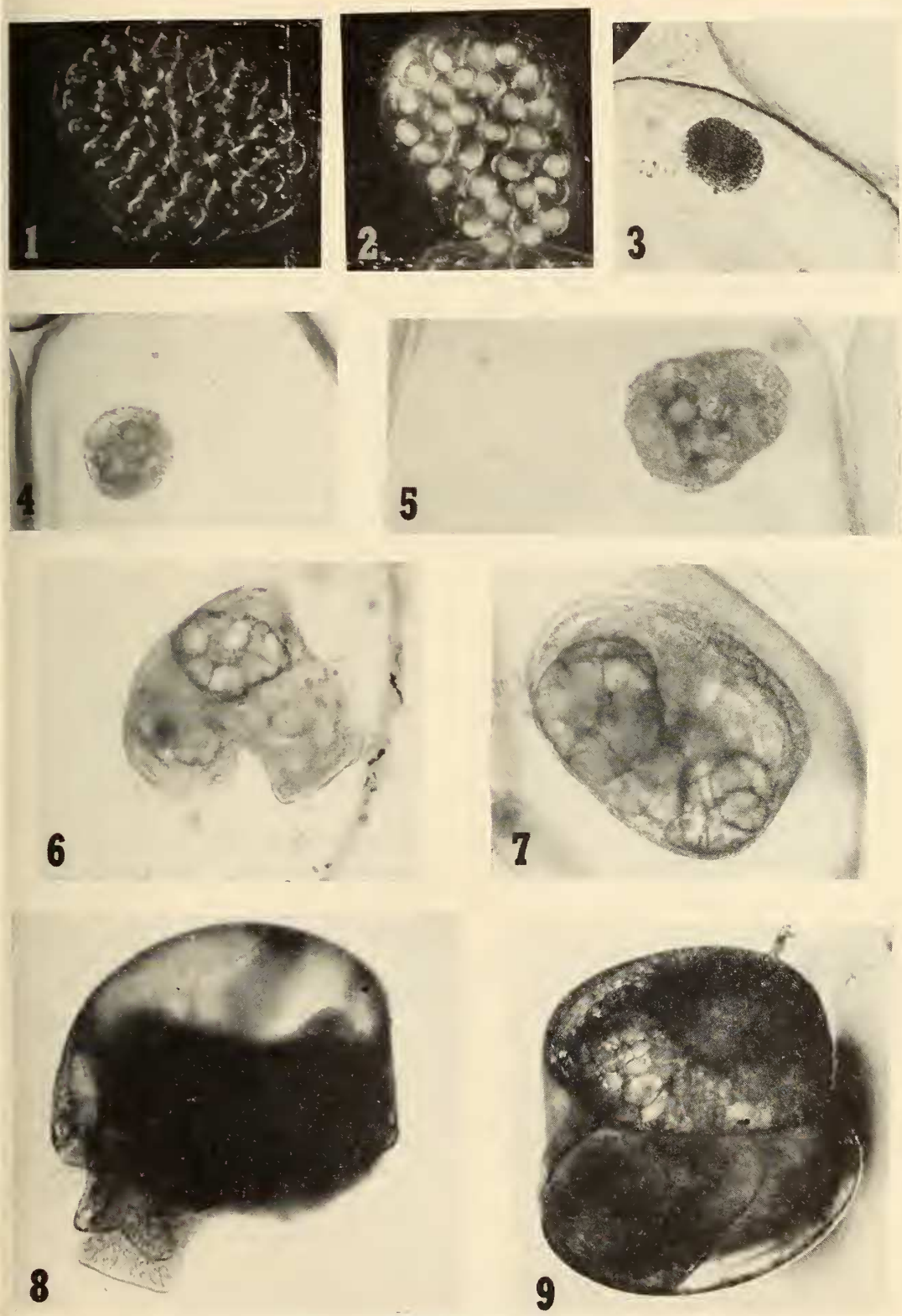

FIG. 1.—(See opposite page for legend).

TABLE 1.—OXYGEN CONSUMPTION OF *AUSTRALORBIS GLABRATUS* EGGS FROM TIME OF DEPOSITION TO HATCHING

Number of experiments	Number of egg-capsules	Number of eggs in a single experiment	Age in days	Mm³ oxygen consumed by one egg in one hour
8	224	811 (367, 992)	0	0.005 (0.004, 0.006)
7	203	774 (589, 866)	1	0.007 (0.006, 0.009)
8	206	703 (494, 992)	2	0.014 (0.011, 0.018)
10	238	582 (367, 872)	3	0.017 (0.014, 0.024)
8	189	554 (315, 877)	4	0.031 (0.028, 0.034)
14	285	467 (280, 752)	5	0.044 (0.035, 0.059)
13	245	438 (254, 752)	6	0.057 (0.048, 0.068)
11	215	464 (266, 752)	7	0.061 (0.049, 0.074)
11	146	357 (283, 504)	8	0.066 (0.054, 0.088)

sumption was determined. In the first series, the same egg clutches were used from 5 to 6 times on alternate or successive days. In view of the rapid development of the ova, care was taken to control the time intervals rigidly, that is, all the experiments started at 9 a.m. Because initially some doubts existed whether the repeated handling of the egg clutches and the shaking in the Warburg flasks might interfere with normal development, a second set was done in which initially a large number of freshly deposited clutches were collected. Of these, certain numbers were selected each day for the experiments (2 to 7 experiments on the various days of development), but they were not used again. The data derived from this second series coincided very closely with those of the first series, both therefore, can be discussed together. The stages reached by the developing embryos were also identical in both series; it is hence justified to assume

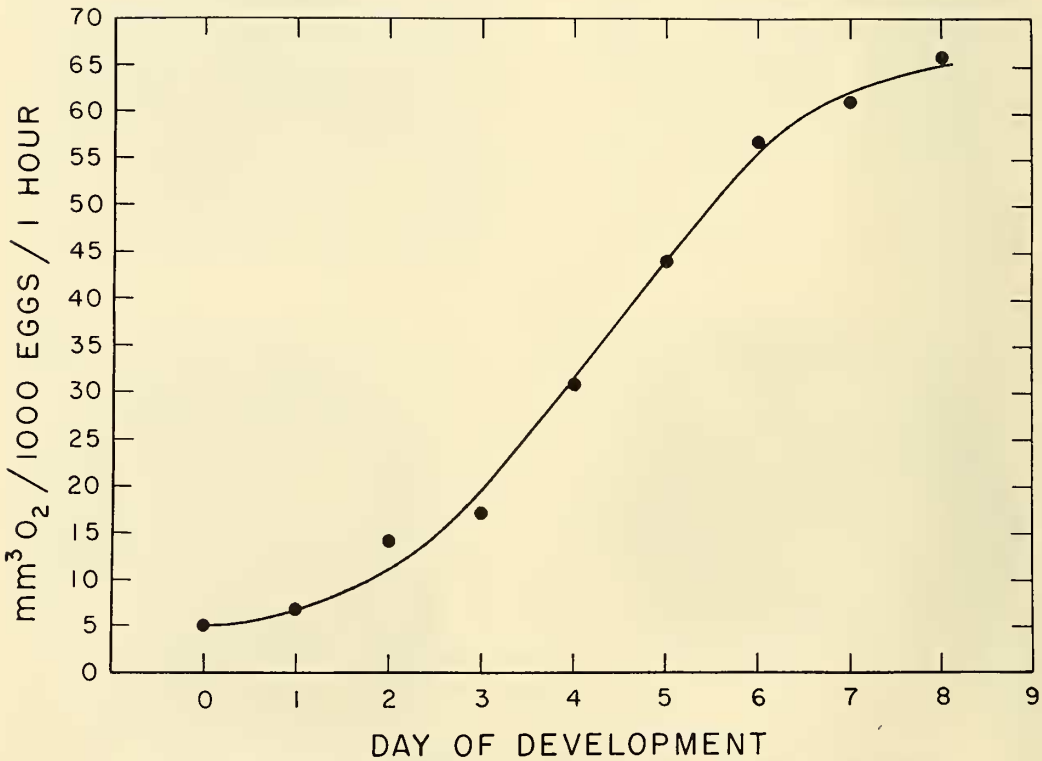

FIG. 2.—Rate of oxygen consumption of developing eggs of *Australorbis glabratus*

that we were dealing with "normal" clutches. This point was further checked by comparing the developmental stages of our experimental clutches with clutches that were not handled at all; we were unable to observe any differences.

Table 1 and Fig. 2 summarize the average results, while Table 2 gives details of the first series. It is evident that the variability in oxygen consumption was relatively small. This is true especially for the very young stages, but even in the older ones uniform results were obtained. This, undoubtedly, was due to the large number of embryos used per Warburg flask. The average values arrange themselves to a smooth curve (Fig. 2) which has an S-shaped form. This is exactly the same type of curve as found during the development of the embryos of higher animals, e.g., hen's egg (Bohr and Hasselbalch, 1900, 1903; Murray, 1925) or turtle egg, (Lynn and von Brand, 1945).

This finding has a bearing on another important point. In working with the type of material as ours, a perennial question is whether the results obtained are really exclusively due to the material one wishes to study, or whether bacterial contaminants obscure the picture. Our egg clutches were certainly not bacteriologically sterile and we cannot exclude categorically slight bacterial contamination. Gross contamination is easily recognized and occurs only when dying eggs are present; as mentioned previously, such clutches were always discarded immediately. It does not seem likely that a smooth curve as shown in Fig. 2 would have been obtained if bacterial respiration had been superimposed to a marked degree on the respiration of the developing snails. Furthermore, in experiments unrelated to the present ones, we tested the respiratory rate of 4 groups of freshly hatched snails, each group comprising 100 to 150 specimens. The egg clutches from which these snails were derived consumed per embryo, just before hatching, 0.064, 0.075, 0.087, and 0.088 mm^3 O_2 per hour. The corresponding values after hatching were 0.086, 0.116, 0.117, and 0.117 mm^3 per young snail per hour. The average value for the embryos was 0.078 mm^3 and for the newly hatched snail 0.109 $mm.^3$ Bacteria would of course not have developed to a marked degree within the developing embryos, but rather in the matrix. If a large number would have been present, one would have expected the respiration of the embryos to have been higher than that of the young snail, while the opposite was true. That the freshly hatched snail should consume somewhat more oxygen than the fully developed embryo is not surprising; the greater activity of the former alone is probably sufficient to explain the difference. We are therefore

TABLE 2.—OXYGEN CONSUMPTION OF DEVELOPING EGGS OF *AUSTRALORBIS GLABRATUS*

Number of egg-capsules in each test from 0–4th day	Number of eggs in each test 0–4th day	Extreme number of eggs in each capsule		Number of egg-capsules in each test from 5th–8th day	Number of eggs in each test 5th–8th day	Mm³ Oxygen consumed by one developing egg in one hour on day								
		min.	max.			0	1	2	3	4	5	5	7	8
30	872	16	42	16	395	0.005	—	—	0.018	—	0.039	—	0.061	0.064
30	851	15	45	20	504	0.005	—	0.016	—	—	0.040	0.055	0.058	0.088*
32	992	14	43	20	410	0.005	—	0.018	—	—	0.041	0.056	0.064	0.073*
30	877	16	30	20	395	0.006	—	—	—	0.031	0.059	0.068	—	0.087*
30	670	12	41	18	350	0.004	—	—	0.017	0.033	—	0.049	0.059	—
18	367	13	40	18	367	0.005	—	—	0.018	—	—	—	—	—
24	589	5	42	18	423	—	0.008	0.014	—	—	0.047	0.057	0.060†	—
30	790	10	45	30	727	—	0.008	0.014	0.024	—	—	—	0.062	—
30	854	12	42	29	752	—	0.006	0.013	—	—	0.043	0.050	0.074†	—
29	689	14	38	28	624	—	0.006	—	—	0.030	0.040	—	0.066†	—
30	680	9	44	30	661	—	—	—	0.020	0.034	0.055	0.065	—	—
20	410	7	34	20	381	—	—	—	0.014	—	0.040	0.058	—	—
20	512	6	43	20	493	—	—	—	0.016	—	0.044	0.061	—	—
20	528	11	45	20	497	—	—	—	0.017	—	0.049	0.062	—	—
20	397	7	30	16	358	—	—	—	—	—	0.042	—	0.067	—

* Many hatched snails attached to the surface of outer capsule.
† After test hatched embryos present in the Warburg flask.

confident that our data truly reflect the respiratory activity of the developing snail.

SUMMARY

The rate of oxygen consumption of the developing egg of *Australorbis glabratus* increases from the time of oviposition to hatching, following a curve strikingly similar to that found in egg-laying vertebrates.

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Vague similarities in certain properties are never sufficient to determine a person who earnestly seeks for the truth and is not shackled by hypotheses.—J. Bergman.